REMARKS

Applicants thank the Examiner for his review of the instant application For the reasons stated below, the rejections of the presently pending claims presented in the Final Office Action dated April 25, 2005, are respectfully traversed. Claims 22-26 are presented for examination.

Status of the Claims

Applicants mailed an Amendment with the Appeal Brief on September 21, 2005, amending Claim 22 to add the term "isolated." The listing of the claims above repeats this amendment as Applicants are unsure of the status of the previously filed Amendment.

Rejection Under 35 U.S.C. §101

The PTO maintains its rejection of pending Claims 22-26 under 35 U.S.C. § 101 as lacking utility for the reasons set forth in the previous Office Actions. The PTO states that the specification discloses that the PRO539 polynucleotide is amplified in lung and colon tumors, and that Applicants have asserted the use of the molecule for diagnosis. However, the PTO rejects this utility, stating that "the overexpression of the nucleic acid is not relevant to the utility of the protein and antibody." *Final Office Action* at 3.

Applicants incorporate by reference their previously submitted arguments, including those made in their Appeal Brief, and for the reasons of record assert that the specification contains a disclosure of utility which corresponds in scope to the subject matter sought to be patented and therefore must be taken as sufficient to satisfy the utility requirement of 35 U.S.C. § 101. Applicants also submit that for reasons of record, the PTO has not met its burden of providing evidence showing that one of ordinary skill in the art would reasonably doubt the asserted utility. However even if the PTO has met its initial burden, Applicants' rebuttal evidence previously submitted and additional evidence submitted herewith is sufficient to prove that it is **more likely than not** that a person of skill in the art would be convinced, **to a reasonable probability,** that the asserted utility is true. As stated previously, Applicants' evidence need not be direct evidence, so long as there is a reasonable correlation between the evidence and the asserted utility. **The standard is not absolute certainty.**

Substantial Utility

Summary of Applicants' Arguments and the PTO's Response

Applicants' asserted utility rests on the following argument:

1. Applicants have provided reliable evidence that gene for the PRO539 polypeptide is amplified by at least two-fold in lung and colon tumors (as explained previously, in Example 16 of the specification, a ΔCt value of 1.0 equals a two-fold amplification, a ΔCt value of 2.0 equals a four-fold amplification, etc.);

2. Applicants assert that it is well-established in the art that amplification of a gene leads to overexpression of the corresponding mRNA, and that a change in the level of mRNA for a particular protein, *e.g.* an increase, generally leads to a corresponding change in the level of the encoded protein, *e.g.* an increase;

3. Given the amplification of the PRO539 gene in lung and colon tumors, it is more likely than not that the PRO539 polypeptide is overexpressed in lung and colon tumors compared to their normal tissue counterparts, making the claimed antibodies useful as diagnostic tools, alone or in combination with other diagnostic tools.

Applicants understand the PTO to be making three arguments in response to Applicants' asserted utility:

1. The PTO challenges the reliability of the evidence reported in Example 16, stating that the amplification was "not even two fold," and that no statistical data were presented;

2. The PTO cites Meric *et al.* (Mol. Cancer Ther. (2002) 1:971-979) Gökman -Polar *et al.* (Cancer Res. (2001) 61:1375-1381) and Pennica *et al.* (Proc. Natl. Acad. Sci. USA (1998) 95:14717-14722) to support its assertion that "not only is there no necessary connection between the level of protein in a cell and the amount of mRNA, but there is also no necessary correlation between the amount of DNA in a cell and the amount of mRNA." *Final Office Action* at 4 (emphasis added). Therefore, the PTO concludes that "any evidence by Applicant showing overexpression of one component does not provide utility for the protein itself." *Id.* at 4-5.

3. Finally, the PTO argues that "given the breadth of these claims which encompass any antibody to the Pro-539 polypeptide, there is an abundance of evidence that very similar proteins can perform very different functions." *Final Office Action* at 5. The PTO cites Rost *et al.* (J. Mol. Biol. (2002) 318(2):595-608) to support the assertion that "even high levels of homology do

not necessarily correlate with actual protein function. In the current case, where the function of PRO-539 (SEQ ID NO: 7) is not known, the expectation is even lower that there is any utility that can be derived based upon the sequence." *Id.*

Applicants respectfully submit that in light of all of the evidence, the PTO's arguments are not adequate to support the utility rejection of the claimed invention under 35 U.S.C. § 101.

The PTO has Acknowledged that the Data Reporting Amplification of the PRO539 Gene is Sufficient to Provide Utility for the PRO539 Nucleic Acids as a Diagnostic Tools

Applicants first address the PTO's argument that the evidence of amplification of the gene encoding the PRO539 polypeptide in lung and colon tumors is less than two-fold, and not statistically significant.

As described in Example 16 of the present application, gene amplification of PRO539 in a variety of primary cancers and cancer cell lines was monitored using real-time quantitative TaqMan™ PCR. The gene amplification results are set forth in Table 7 (Table 8 as amended) on page 117 of the specification. As explained in the specification on page 112, lines 17-19, the results of TaqMan™ PCR are reported in ΔCt units. It is well-known in the art that "Ct" stands for "threshold cycle." **One Ct unit corresponds to one PCR cycle or approximately a 2-fold amplification**, relative to control, 2 units correspond to 4-fold amplification, 3 units to 8-fold amplification, *etc. Specification* at 112, lines 17-19. Looking at the results reported on page 117, nine primary lung tumors and eight primary colon tumors were tested, as well as a number of tumor cell lines. PRO539 had a ΔCt value of greater than 1, *i.e.*, more than two-fold amplification, in six of nine lung tumors and five of eight colon tumors. These data show that in more than half of the lung and colon tumors tested, the gene for PRO539 was amplified at least two-fold. Thus, the PTO's statement that the overexpression of PRO539 was minimal, "not even a two fold overexpression in any cell type" is simply wrong.

Applicants also note that the fact that not all tumors show amplification does not mean that PRO539 cannot be used as a diagnostic or prognostic tool. Press *et al.* (J. Clin. Oncology 2002; 20(14):3095-3105) (attached as Exhibit 1), state that "HER-2/*neu* (c-*erb*B-2) gene amplification, identified in 20% to 30% of breast cancers, is a prognostic marker of poor clinical outcome in node-negative and node-positive breast cancer...." *Id.* at 3095, col. 1., ¶ 2 (emphasis

added). In the instant case, greater than 50% of lung and colon tumors tested showed gene amplification.

The PTO has also rejected the data because it questions the statistical significance of the data. However, Applicants are not required to prove utility to a statistical certainty, only that it is more likely than not true. *See Nelson v. Bowler*, 626 F.2d 853, 856-57, 206 U.S.P.Q. 881, 883-84 (C.C.P.A. 1980) (reversing the Board and rejecting an argument that evidence of utility was insufficient because it was not statistically significant). As the M.P.E.P. states:

> [T]he applicant does not have to provide evidence sufficient to establish that an asserted utility is true "beyond a reasonable doubt." **Nor must the applicant provide evidence such that it establishes an asserted utility as a matter of statistical certainty**. Instead, evidence will be sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely than not true. *M.P.E.P.* § 2107.02, part VII (bold emphasis added, underline in original, citations omitted).

Therefore, whether the results are statistically significant or not is irrelevant to establishing the asserted utility. The results must simply be reliable enough that one of skill in the art would believe that the utility is more likely than not true.

Applicants note that in the closely related application Serial No. 10/033,167, directed to nucleic acids related to SEQ ID NO:6 which encodes the PRO539 polypeptide, the PTO has acknowledged that the nucleic acids have utility. *See Notice of Allowability for Application 10/033,167 dated 7/21/2005*. In that case, the exact same data from Example 16 was relied on for utility of the claimed nucleic acids as diagnostic tools for lung and colon tumors, and the PTO made the same arguments regarding the insufficiency of the data in Example 16. *See Office Action for Application 10/033,167 dated 4/28/03* at 4. In response to Applicants' arguments to the contrary, and the Declaration of Audrey Goddard, the PTO stated "The rejection of Claims 22-41 under 35 U.S.C. § 101 is withdrawn in view of the Declaration" *See Office Action for Application 10/033,167 dated 9/9/03* at 2. Therefore, Applicants submit that the PTO's rejection of the exact same data in the instant case based on the same arguments of alleged insufficient details are moot in light of this statement. As such, the data in Example 16 are sufficient to establish utility for the PRO539 nucleic acids as a diagnostic tool.

In conclusion, Applicants submit that the evidence reported in Example 16, supported by the Goddard Declaration, establish that there is at least a two-fold amplification of the PRO539

gene in a majority of the lung and colon tumors tested. The PTO has accepted that the data in Example 16 are sufficient to establish utility for the nucleic acids encoding the PRO539 polypeptide as diagnostic tools, and therefore any challenge to the sufficiency of the data with respect to the utility of the nucleic acid is inappropriate. Therefore, the only issue which remains is whether the data in Example 16 regarding amplification of the PRO539 gene are reasonably correlated with overexpression of the PRO539 mRNA and polypeptide such that the antibodies to the PRO539 polypeptide have utility as diagnostic tools as well. As discussed below, even if the PTO has established a reasonable doubt regarding Applicants' assertion that they are reasonably correlated, Applicants' overwhelming rebuttal evidence is more than sufficient to establish that changes in mRNA level more often than not lead to corresponding changes in protein level.

The PTO's Evidence Does Not Establish a Reasonable Basis to Doubt the Asserted Utility

Applicants turn next to the second portion of their argument in support of their asserted utility – that it is well-established in the art that amplification of a gene leads to overexpression of the mRNA and protein; given Applicants' evidence of amplification of the PRO539 gene in lung and colon tumors, it is likely that the PRO539 mRNA and polypeptide are also differentially expressed; and antibodies to proteins differentially expressed in certain tumors have utility as diagnostic tools.

In response to Applicants' assertion, the PTO cites Meric *et al.* (Mol. Cancer Ther. (2002) 1:971-979) Gökman -Polar *et al.* (Cancer Res. (2001) 61:1375-1381) and Pennica *et al.* (Proc. Natl. Acad. Sci. USA (1998) 95:14717-14722) to support its assertion that "not only is there no necessary connection between the level of protein in a cell and the amount of mRNA, but there is also no necessary correlation between the amount of DNA in a cell and the amount of mRNA." *Final Office Action* at 4 (emphasis added).

Applicants have previously discussed at length why the Meric, Gökman -Polar and Pennica references do not support the PTO's position. Applicants incorporate by reference the previous arguments, including those in the Appeal Brief.

Briefly stated, Applicants argue that Meric supports Applicants' assertion that generally, changes in mRNA lead to a corresponding change in the level of the encoded protein – that is

why examining differences between tumor and normal tissue at the mRNA level is a "fundamental principle" of molecular cancer therapeutics. Likewise, Meric teaches that mRNA overexpression can be attributed to gene amplification. Gökman-Polar reports only that increased protein levels were not accompanied by increased mRNA levels – this is not contrary to Applicants' assertion that gene amplification leads to overexpression of an mRNA which leads to overexpression of the corresponding protein. In addition, Gökman-Polar reports a positive correlation between changes in mRNA level and changes in protein level for five of six samples tested. Finally, Pennica, the only reference which looked at a correlation between gene amplification and gene expression, reports one gene where there was a strong correlation between the two, and one possible example where there was a lack of positive correlation. This evidence is at best inconclusive, with at least half the genes showing a correlation between gene amplification and mRNA overexpression.

While these references possibly establish that there is no "necessary" correlation between gene amplification and overexpression of mRNA and protein, Applicants are not required to establish the asserted utility beyond a reasonable doubt or to a statistical certainty. Thus, even assuming that the PTO has proved that there is no "necessary" correlation, this does not mean that it has met its initial burden of establishing that it is "more likely than not" that a skilled artisan would doubt the asserted utility. Given that two of the cited references actually support the Applicants' position, Applicants assert that the PTO has failed to meet its burden of establishing a *prima facie* case of lack of utility.

Finally, Applicants address the PTO's argument that "there is an abundance of evidence that very similar proteins can perform very different functions" and that "even high levels of homology do not necessarily correlate with actual protein function. In the current case, where the function of PRO-539 (SEQ ID NO: 7) is not known, the expectation is even lower that there is any utility that can be derived based upon the sequence." *Final Office Action* at 5.

Applicants' asserted utility does not rely on the function of the PRO539 protein, or any relation between the function of PRO539 and its sequence. The claimed subject matter relates to antibodies which specifically bind to the polypeptide of SEQ ID NO:7. The Applicants' asserted utility is the use of the claimed antibodies as diagnostic tools for cancer, based on the two-fold amplification of the PRO539 gene in a majority of the lung and colon tumors tested. This utility

in no way depends on the function of the PRO539 protein, making the PTO's arguments irrelevant.

In summary, Applicants assert that for the reason of record, the PTO's evidence does not establish a reasonable basis for one of skill in the art to doubt the asserted utility, and therefore the PTO has failed to meet its burden of establishing a *prima facie* case of lack of utility.

Applicants' Evidence Establishes that a Amplification of a Gene Leads to Overexpression of the Corresponding mRNA

In support of the assertion that gene amplification results in overexpression of the corresponding mRNA, Applicants previously submitted the Declaration of Victoria Smith, an expert in the field, excerpts from Genes V, a leading textbook in the field (Benjamin Lewin, Genes V, 5th ed. 1994, pages 1196-1201), and references by Alitalo (Med. Biol., 1984; 62:304-317), Merlino *et al.* (J. Clin. Invest., 1985; 75:1077-1079), Orntoft *et al.* (Molecular and Cellular Proteomics, 2002; 1:37-45), Hyman *et al.* (Cancer Research, 2002; 62:6240-6245), Pollack *et al.* (PNAS, 2002; 99:12963-12968), Bahnassy *et al.* (BMC Gastroenterology, 2004; 4:22-34), and Blancato *et al.* (British Journal of Cancer, 2004; 90(8):1612-1619). The details of the teachings of these declarations and references, and how they support Applicants' asserted utility, are of record and will not be repeated here.

In addition to the supporting references previously submitted by Applicants, Applicants submit the following references to further support the assertion that gene amplification results in overexpression of the corresponding mRNA.

In a study by Shiga *et al.* (Anticancer Res. 1993; 13(5A):1293-301) (Abstract attached as Exhibit 2), the authors examined amplification and expression of the c-erbB-2 protooncogene in 60 primary human esophageal cancers and 12 cell lines established from the cancers. *Id.* at Abstract. Amplification was found in 4 out of 60 cancer samples and 1 out of 12 cell lines. The authors report that "[o]verexpression of c-erbB-2 gene product, as determined by both Northern blot hybridization [*i.e.* mRNA] and immunohistochemical analysis [*i.e.* protein], was found in the cells with c-erbB-2 amplification, but not in normal esophageal mucosae or in primary esophageal carcinomas without gene amplification." *Id.* This supports the Applicants' assertion that gene amplification leads to overexpression of both the corresponding mRNA and protein.

Riou *et al.* (Mol. Carcinog. 1995; 12(3):124-31) (Abstract attached as Exhibit 3) tested 25 testicular germ-cell (TGC) tumors mdm-2 amplification and gene expression. *Id.* at Abstract. The authors report that "mdm-2 gene amplification (2.5- to 10-fold) was detected in three (12%) of these TGC tumors. These three tumors, and eight additional TGC tumors, overexpressed mdm-2 mRNA." *Id.* This reference teaches that while not the only cause of mRNA overexpression, gene amplification leads to mRNA overexpression.

In a paper reporting on the amplification and overexpression of the DEAD box gene, DDX1, in retinoblastoma (RB) and neuroblastoma (NB) cell lines, Godbout *et al.* (J. Biol. Chem. 1998; 273(33):21161-8) (Abstract attached as Exhibit 4), the authors report "there is a good correlation with DDX1 gene copy number, DDX1 transcript levels, and DDX1 protein levels in all cell lines studied." *Id.* at Abstract. This reference, published in 1998, clearly shows that one of skill in the art would have a reasonable basis to believe that gene amplification leads to overexpression of the corresponding mRNA and protein.

Similarly, Bea *et al.* (Cancer Res. 2001; 61(6):2409-12) (Abstract attached as Exhibit 5) examined the level of gene copy number, transcript level, and protein level of BMI-1 in several cancers. The authors report finding gene amplification in 4 of 36 mantle cell lymphomas (MCLs). *Id.* at Abstract. The authors state that "[t]he four tumors with gene amplification showed significantly higher mRNA levels than other MCLs and NHLs with the BMI-1 gene in germline configuration. Five additional MCLs also showed very high mRNA levels without gene amplification. A good correlation between BMI-1 mRNA levels and protein expression was observed in all types of lymphomas." *Id.* As mentioned above, the fact that mechanisms other than amplification lead to mRNA overexpression does not detract from the fact that the reference supports Applicants' assertion that gene amplification leads to overexpression of the corresponding mRNA and protein.

In a more recent article, Benetkieicz *et al.* (Genes Chromosomes Cancer. 2005; 42(3):228-37) (Abstract attached as Exhibit 6) examined DNA copy number and gene expression of 22q in 18 ovarian cancers. The authors report that they were able to identify "21 deleted genes showing low mRNA levels and 12 amplified genes displaying elevated gene expression." *Id.* at Abstract. Based on these data, the authors concluded that "[o]ur results indicated significant correlation between DNA copy number aberrations and variation in mRNA expression." *Id.*

Thus, this reference supports not only the narrow assertion that gene amplification leads to overexpression, but the broad concept that gene copy number, whether increased or decreased, correlates with mRNA level.

In addition, in the reference by Press *et al.* discussed above (Exhibit 1), the authors state that "we have shown a correlation between gene amplification and an increased level of HER-2/*neu* expression, referred to as overexpression, at the mRNA and protein product levels." *Press et al.* at 3095, col. 1, ¶ 3.

These studies are representative of numerous published studies which support Applicants' assertion that gene amplification leads to overexpression of the corresponding mRNA. Applicants submit herewith an additional 8 references (abstracts attached as Exhibit 7) which support Applicants' assertion. While Applicants have previously acknowledged that the correlation between gene amplification and mRNA overexpression is not a "necessary" or exact one, (*see, e.g.,* abstracts attached as Exhibit 8), Applicants remind the PTO that the asserted utility does not have to be established to a statistical certainty, or beyond a reasonable doubt. *See M.P.E.P.* at § 2107.02, part VII (2004). Therefore, the fact that there are exceptions to the general principle that there is a correlation between gene amplification and mRNA overexpression does not provide a proper basis for rejecting Applicants' asserted utility.

Applicants' Evidence Establishes that a Change in mRNA Level for a Particular Gene lead to Corresponding Change in the Level of the Encoded Protein

In support of the assertion that changes in mRNA, *e.g.* an increase, are positively correlated to changes in protein levels, Applicants previously submitted a copy of a Declaration by J. Christopher Grimaldi, a copy of the declaration of Paul Polakis, Ph.D., excerpts from the Molecular Biology of the Cell, a leading textbook in the field (Bruce Alberts, *et al.*, Molecular Biology of the Cell (3rd ed. 1994) and (4th ed. 2002), excerpts from the textbook, Genes VI, (Benjamin Lewin, Genes VI (1997)), a reference by Zhigang *et al.*, (World Journal of Surgical Oncology 2004; 2:13-20, and a reference by Meric *et al.* (Molecular Cancer Therapeutics, 2002; 1:971-979). The details of the teachings of these declarations and references, and how they support Applicants' asserted utility, are of record and will not be repeated here.

However, without acquiescing to the PTO's criticism that Dr. Polakis' Declaration does not provide "data such that the examiner can independently draw conclusions," Applicants submit herewith a copy of a second Declaration by Dr. Polakis (attached as Exhibit 9) that presents evidentiary data in Exhibit B. Exhibit B of the Declaration identifies 28 gene transcripts out of 31 gene transcripts (i.e., greater than 90%) that showed good correlation between tumor mRNA and tumor protein levels. As Dr. Polakis' second Declaration says "[a]s such, in the cases where we have been able to quantitatively measure both (i) mRNA and (ii) protein levels in both (i) tumor tissue and (ii) normal tissue, we have observed that in the vast majority of cases, there is a very strong correlation between increases in mRNA expression and increases in the level of protein encoded by that mRNA." Accordingly, Dr. Polakis has provided the facts to enable the Examiner to draw independent conclusions.

The case law has clearly established that in considering affidavit evidence, the Examiner must consider all of the evidence of record anew. *See in re Rinehart,* 531 F.2d 1084, 189 USPQ 143 (C.C.P.A. 1976); *In re Piasecki,* 745 F.2d. 1015, 226 USPQ 881 (Fed. Cir. 1985). "After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of the evidence with due consideration to persuasiveness of argument." *In re Alton,* 37 U.S.P.Q.2d 1578, 1584 (Fed. Cir. 1996), *quoting In re Oetiker,* 977 F.2d 1443, 1445, 24 U.S.P.Q.2d 1443, 1444 (Fed. Cir. 1992). Furthermore, the Federal Court of Appeals held in *In re Alton*, "We are aware of no reason why opinion evidence relating to a fact issue should not be considered by an examiner." *Id.* at 1583. Applicants also respectfully draw the PTO's attention to the Utility Examination Guidelines which state, "Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered." *66 Fed. Reg. 1098, Part IIB (2001).*

In addition to the supporting references previously submitted by Applicants, Applicants submit the following references to further support the assertion that changes in mRNA levels generally lead to corresponding changes in the level of the encoded polypeptide.

In a comprehensive study by Ornstoft *et al.* (Mol. Cell. Proteomics. 2002; 1(1):37-45) (previously submitted), the authors examined gene amplification, mRNA expression level, and

protein expression in pairs of non-invasive and invasive human bladder tumors. *Id.* at Abstract. The authors examined 40 well resolved abundant known proteins, and found that "[i]n general there was a highly significant correlation ($p<0.005$) between mRNA and protein alterations. Only one gene showed disagreement between transcript alteration and protein alteration." *Id.* at 42, col. 2. Clearly, a correlation in 39 of 40 genes examined supports Applicants' assertion that changes in mRNA level generally lead to corresponding changes in protein level.

In a study by Wang *et al.* (Urol. Res. 2000; 28(5):308-15) (abstract attached as Exhibit 10) the authors report that down-regulation of E-cadherin protein has been shown in various human tumors. *Id.* at Abstract. In the reported study, the authors examined the expression of cadherins and associated catenins at the mRNA level in paired tumor and nonneoplastic primary prostate cultures. They report that "[s]ix of seven cases of neoplastic cultures showed moderately-to-markedly decreased levels of E-cadherin and P-cadherin mRNA. Similar losses of alpha-catenin and beta-catenin mRNA were also observed." *Id.* As Applicants' assertion would predict, the authors state that the mRNA measures showed "good correlation" with the results from protein measures. The authors conclude by stating that "this paper presents a coordinated down-regulation in the expression of E-cadherin and associated catenins at the mRNA and protein level in most of the cases studied." *Id.*

In a more recent study by Munaut *et al.* (Int. J. Cancer. 2003; 106(6):848-55) (abstract attached as Exhibit 11) the authors report that vascular endothelial growth factor (VEGF) is expressed in 64-95% of glioblastomas (GBMs), and that VEGF receptors (VEGFR-1, its soluble form sVEGFR-1, VEGFR-2 and neuropilin-1) are expressed predominantly by endothelial cells. *Id.* at Abstract. The authors explain that infiltrating tumor cells and newly-formed capillaries progress through the extracellular matrix by local proteolysis involving matrix metalloproteinases (MMPs). In the present study, the authors "used quantitative RT-PCR, Western blot, gelatin zymography and immunohistochemistry to study the expression of VEGF, VEGFR-1, VEGFR-2, sVEGFR-1, neuropilin-1, MT1-MMP, MMP-2, MMP-9 and TIMP-2 in 20 human GBMs and 5 normal brains. The expression of these MMPs was markedly increased in most GBMs with excellent correlation between mRNA and protein levels." *Id.* Thus, the results support Applicants' assertion that changes in mRNA level lead to corresponding changes in protein level.

In another recent study, Hui *et al.* (Leuk. Lymphoma. 2003; 44(8):1385-94 (abstract attached as Exhibit 12) used real-time quantitative PCR and immunohistochemistry to evaluate cyclin D1 mRNA and protein expression levels in mantle cell lymphoma (MCL). *Id.* at Abstract. The authors report that seven of nine cases of possible MCL showed overexpression of cyclin D1 mRNA, while two cases showed no cyclin D1 mRNA increase. *Id.* Similarly, "[s]ix of the seven cyclin D1 mRNA overexpressing cases showed increased cyclin D1 protein on tissue array immunohistochemistry; one was technically suboptimal." *Id.* The authors conclude that the study "demonstrates good correlation and comparability between measure of cyclin D1 mRNA ... and cyclin D1 protein." *Id.* Thus, this reference supports Applicants' assertion.

In a recent study by Khal *et al.* (Int. J. Biochem. Cell Biol. 2005; 37(10):2196-206) (abstract attached as Exhibit 13) the authors report that atrophy of skeletal muscle is common in patients with cancer and results in increased morbidity and mortality. *Id.* at Abstract. To further understand the underlying mechanism, the authors studied the expression of the ubiquitin-proteasome pathway in cancer patient muscle using a competitive RT-PCR to measure expression of mRNA for proteasome subunits C2 and C5, while protein expression was determined by western blotting. "Overall, both C2 and C5 gene expression was increased by about three-fold in skeletal muscle of cachectic cancer patients (average weight loss 14.5+/-2.5%), compared with that in patients without weight loss, with or without cancer. ... There was a good correlation between expression of proteasome 20Salpha subunits, detected by western blotting, and C2 and C5 mRNA, showing that increased gene expression resulted in increased protein synthesis." These findings support Applicants' assertion that changes in mRNA level lead to changes in protein level.

Maruyama *et al.* (Am. J. Patho. 1999; 155(3):815-22) (abstract attached as Exhibit 14) investigated the expression of three Id proteins (Id-1, Id-2 and Id-3) in normal pancreas, in pancreatic cancer and in chronic pancreatitis (CP). The authors report that pancreatic cancer cell lines frequently coexpressed all three Ids, "exhibiting good correlation between Id mRNA and protein levels." *Id.* at Abstract. In addition, the authors teach that all three Id mRNA levels were expressed at high levels in pancreatic cancer samples compared to normal or CP samples. At the protein level, Id-1 and Id-2 staining was faint in normal tissue, while Id-3 ranged from weak to strong. In contrast, in the cancer tissues "many of the cancer cells exhibited abundant Id-1, Id-2,

and Id-3 immunoreactivity," and Id-1 and Id-2 protein was increased significantly in the cancer cells by comparison to the respective controls, mirroring the overexpression at the mRNA level. Thus, the authors report that in both cell lines and tissue samples, increased mRNA levels leads to an increase in protein overexpression, supporting Applicants' assertion.

Support for Applicants' assertion is also found in an article by Caberlotto *et al.* (Neurosci. Lett. 1999; 256(3):191-4) (abstract attached as Exhibit 15). In a previous study, the authors investigated alterations of neuropeptide Y (NPY) mRNA expression in the Flinders Sensitive Line rats (FSL), an animal model of depression. *Id.* at Abstract. The authors reported that in the current study, that NPY-like immunoreactivity (NPY-LI) was decreased in the hippocampal CA region, and increased in the arcuate nucleus, and that fluoxetine treatment elevated NPY-LI in the arcuate and anterior cingulate cortex. The authors state that "[t]he results demonstrate a good correlation between NPY peptide and mRNA expression." Thus, increases and decreases in mRNA levels were reflected in corresponding changes in protein level.

Misrachi and Shemesh (Biol. Reprod. 1999; 61(3):776-84) (abstract attached as Exhibit 16) investigated their hypothesis that FSH regulates the bovine cervical prostaglandin E(2) (PGE(2)) synthesis that is known to be associated with cervical relaxation and opening at the time of estrus. *Id.* at Abstract. Cervical tissue from pre-estrous/estrous, luteal, and postovulatory cows were examined for the presence of bovine (b) FSH receptor (R) and its corresponding mRNA. The authors report that bFSHR mRNA in the cervix was maximal during pre-estrus/estrus, and that the level of FSHR protein was significantly higher in pre-estrous/estrous cervix than in other cervical tissues. *Id.* The authors state that "[t]here was a good correlation between the 75-kDa protein expression and its corresponding transcript of 2.55 kb throughout the estrous cycle as described by Northern blot analysis as well as RT-PCR." *Id.* Thus, changes in the level of mRNA for bFSHR led to corresponding changes in FSHR protein levels, a result which supports Applicants' assertion.

In a study by Stein *et al.* (J. Urol. 2000; 164(3 Pt 2):1026-30) (abstract attached as Exhibit 17), the authors studied the role of the regulation of calcium ion homeostasis in smooth muscle contractility. *Id.* at Abstract. The authors investigated the correlation between sarcoplasmic endoplasmic reticulum, calcium, magnesium, adenosine triphosphatase (SERCA) protein and gene expression, and the contractile properties in the same bladder. Partial bladder outlet

obstructions were created in adult New Zealand white rabbits, which were divided into control, sham operated and obstructed groups. Stein *et al.* report that "[t]he relative intensities of signals for the Western [protein] and Northern [mRNA] blots demonstrated a strong correlation between protein and gene expression. ... The loss of SERCA protein expression is mediated by down-regulation in gene expression in the same bladder." *Id.* This report supports Applicants' assertion that changes in mRNA level, e.g. a decrease, lead to a corresponding change in the level of the encoded protein, e.g. a decrease.

In an article by Gou and Xie (Zhonghua Jie He He Hu Xi Za Zhi. 2002; 25(6):337-40) (abstract attached as Exhibit 18) the authors investigated the expression of macrophage migration inhibitory factor (MIF) in human acute respiratory distress syndrome(ARDS) by examining the expression of MIF mRNA and protein in lung tissue in ARDS and normal persons. *Id.* at Abstract. The authors report "undetectable or weak MIF mRNA and protein expression in normal lungs. In contrast, there was marked upregulation of MIF mRNA and protein expression in the ARDS lungs." *Id.* This is consistent with Applicants' assertion that a change in mRNA for a particular gene, e.g. an increase, generally leads to a corresponding change in the level of protein expression, e.g. an increase.

These studies are representative of numerous published studies which support Applicants' assertion that changes in mRNA level generally lead to corresponding changes in the level of the expressed protein. Applicants submit herewith an addition 70 references (abstracts attached as Exhibit 19) which support Applicants' assertion. In addition, Applicants note that Exhibits 1, 2, 4, 5, and several of the references submitted as Exhibit 7, also report a correlation between mRNA overexpression resulting from gene amplification and overexpression of the corresponding protein. *See discussion supra.*

In addition to these supporting references, Applicants also submit herewith additional references which offer indirect support of Applicants' asserted utility. These reference report a general correlation between mRNA and protein levels, contrary to Gökman-Polar, the PTO's only reference which examined the relationship between mRNA and protein levels.

For example, in an article by Futcher *et al.* (Mol. Cell Biol. 1999; 19(11):7357-68) (abstract attached as Exhibit 20) the authors conducted a study of mRNA and protein expression

in yeast. Contrary to the results of an earlier study by Gygi *et al.*, Futcher *et al.* report "a good correlation between protein abundance, mRNA abundance, and codon bias." *Id.* at Abstract.

Similarly, in an article by Papotti *et al.* (Virchows Arch. 2002; 440(5):461-75) (abstract attached as Exhibit 21) the authors examined the expression of three somatostatin receptors (SSTR) at the mRNA and protein level in forty-six tumors. *Id.* at Abstract. The authors report a "good correlation between RT-PCR [mRNA level] and IHC [protein level] data on SSTR types 2, 3, and 5." *Id.*

Van der Wilt *et al.* (Eur. J. Cancer. 2003; 39(5):691-7) (abstract attached as Exhibit 22) studied deoxycytidine kinase (dCK) in seven cell lines, sixteen acute myeloid leukemia samples, ten human liver samples, and eleven human liver metastases of colorectal cancer origin. *Id.* at Abstract. The authors report that "enzyme activity and protein expression levels of dCK in cell lines were closely related to the mRNA expression levels" and that there was a "good correlation between the different dCK measurements in malignant cells and tumors." *Id.*

Grenback *et al.* (Regul. Pept. 2004; 117(2):127-39) (abstract attached as Exhibit 23) studied the level of galanin in human pituitary adenomas using a specific radioimmunoassay. *Id.* at Abstract. The authors report that "[i]n the tumors analyzed with in situ hybridization there was a good correlation between galanin peptide levels and galanin mRNA expression." *Id.*

Similarly, Shen *et al.* (Blood. 2004; 104(9):2936-9) (abstract attached as Exhibit 24) examined the level of B-cell lymphoma 2 (BCL2) protein expression in germinal center (GC) B-cells and diffuse large B-cell lymphoma (DLBCL). *Id.* at Abstract. The authors report that "GC cells had low expression commensurate with the low protein expression level" and that in DLBCL the level of BCL2 mRNA and protein expression showed "in general, a good correlation." *Id.*

Likewise, in an article by Fu *et al.* (Blood 2005; 106(13):4315-21) (abstract attached as Exhibit 25) the authors report that six mantle cell lymphomas studied "expressed either cyclin D2 (2 cases) or cyclin D3 (4 cases)." *Id.* at Abstract. "There was a good correlation between cyclin D protein expression and the corresponding mRNA expression levels by gene expression analysis." *Id.*

These examples are only a few of the many references Applicants could cite in rebuttal to the PTO's arguments. Applicants submit herewith 26 additional references (abstracts attached as

Exhibit 26) which also support Applicants' assertion in that the references report a correlation between the level of mRNA and corresponding protein, contrary to the assertion of the PTO that mRNA and protein levels are not correlated.

In summary, Applicants submit herewith a total of 112 references in addition to the declarations and references already of record which support Applicants' asserted utility, either directly or indirectly. These references support the assertion that in general, a change in mRNA expression level for a particular gene leads to a corresponding change in the level of expression of the encoded protein. As Applicants have previously acknowledged, the correlation between changes in mRNA level and protein level is not a "necessary" or exact one, and there are exceptions (*see, e.g.,* abstracts attached as Exhibit 27). However, Applicants remind the PTO that the asserted utility does not have to be established to a statistical certainty, or beyond a reasonable doubt. *See M.P.E.P.* at § 2107.02, part VII (2004). Therefore, the fact that there are exceptions to the correlation between changes in mRNA and changes in protein does not provide a proper basis for rejecting Applicants' asserted utility.

Substantial Utility - Conclusion

In conclusion, Applicants submit that they have offered sufficient evidence to establish that it is more likely than not that one of skill in the art would believe that because the PRO539 gene is amplified in lung and colon tumors, the PRO539 mRNA and polypeptide will be overexpressed in lung and colon tumors. This overexpression of the PRO539 polypeptide makes the claimed antibodies useful as diagnostic tools for cancer, particularly lung and colon cancer. Applicants submit that considering the evidence as a whole, with the overwhelming majority of the evidence supporting Applicants' asserted utility, a person of skill in the art would conclude that Applicants' asserted utility is "more likely than not true." *Id.*

Specific Utility

The Asserted Substantial Utilities are Specific to the Claimed Antibodies

The PTO argues that even if substantial utility were found, there is no specific utility given for antibodies to the PRO539 protein, since antibodies to the protein, as distinguished from

the nucleic acid, have not been associated with any disease, condition, or any other specific feature.

Specific utility is defined as utility which is "specific to the subject matter claimed," in contrast to "a general utility that would be applicable to the broad class of the invention." M.P.E.P. § 2107.01, part I (2004). Applicants submit that the evidence of amplification and overexpression of PRO539 nucleic acids in certain types of cancer cells along with the declarations and references discussed above provide a specific utility for the claimed antibodies. As stated above, Applicants have established a reasonable correlation between gene amplification, gene overexpression, and protein overexpression. This makes antibodies to the PRO539 protein useful in diagnosing lung and colon cancer. This is not a general utility that would apply to the broad class of antibodies.

Utility – Conclusion

Applicants remind the PTO that the evidence supporting utility does not need to be direct evidence, nor does it need to provide a "necessary" or an exact correlation between the submitted evidence and the asserted utility. Instead, evidence which is "reasonably" correlated with the asserted utility is sufficient. *See Fujikawa v. Wattanasin*, 93 F.3d 1559, 1565, 39 U.S.P.Q. 2d 1895 (Fed. Cir. 1996) ("a 'rigorous correlation' need not be shown in order to establish practical utility; 'reasonable correlation' suffices"); *Cross v. Iizuka*, 753 F.2d 1040, 1050, 224 U.S.P.Q. 739 (Fed. Cir. 1985) (same); *Nelson v. Bowler*, 626 F.2d 853, 857, 206 U.S.P.Q. 881 (C.C.P.A. 1980) (same). In addition, utility need only be shown to be "more likely than not true," not to a statistical certainty. *M.P.E.P.* at § 2107.02, part VII (2004). Considering the evidence as a whole in light of the relevant standards for establishing utility, Applicants have established at least one specific, substantial, and credible utility. In view of the above, Applicants respectfully request that the PTO reconsider and withdraw the utility rejection under 35 U.S.C. §101.

Rejection under 35 U.S.C. §112 – Enablement

The PTO rejected Claims 22-27 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. The PTO cites *In re Wands* and the factors set forth

therein to determine the scope of enablement. The PTO's arguments are largely the same as those for the utility rejection: "With regard to enablement, fundamentally the same arguments [as those given for utility] apply, and this rejection is maintained for the same reasons as given above in response to the arguments on utility." *Final Office Action* at 15.

For the reasons of record, including those articulated in Applicants' Appeal Brief, Applicants submit that the claimed antibodies are enabled, as one of skill in the art would know how to make and use them. Applicants submit that the evidence, declarations, references, and arguments discussed above make clear that Applicants have established that it is more likely than not that one of skill in the art would be convinced, to a reasonable probability, that the PRO539 protein is overexpressed in certain cancers, and therefore antibodies to PRO539 have utility as a diagnostic tool. To the extent that the enablement rejection is based on a lack of utility, Applicants respectfully request that the PTO reconsider and withdraw the enablement rejection under 35 U.S.C. §112.

As to the PTO's recitation of the *In re Wands* factors, Applicants note that the question of enablement regarding antibodies is the very issue that was addressed in *In re Wands*, 858 F.2d 731, 8 U.S.P.Q. 2d 1400 (Fed. Cir. 1988). In *Wands*, the CAFC held that the disclosure was sufficient to enable one of skill in the art to make monoclonal antibodies to a disclosed antigen without undue experimentation. *Id.* at 740. If the disclosure was sufficient at the time of filing of the *Wands* application in 1980, it cannot be that the art of making antibodies has become less predictable in the ensuing 25 years, and now requires undue experimentation.

In addition, Applicants submit that the specification discloses how to make and use the claimed antibodies. For example, Example 27 on page 127 of the specification specifically describes the preparation of antibodies that bind PRO polypeptides. *Specification* at 90, line 20 through 97, line 4, and 127, line 13, through 128, line 1. The specification also discloses that the claimed antibodies can be used in diagnostic assays to detect the expression of PRO539 in specific types of tissue. *Specification* at 98, lines 5-29.

Therefore, given the teaching in the specification on how to make and use the claimed antibodies to detect expression of PRO539 in specific tissues, one of skill in the art would be enabled to practice the claimed invention without undue experimentation. Thus, at least one use of antibodies to the PRO539 polypeptide is adequately enabled, which is all that is required – "if

any use is enabled when multiple uses are disclosed, the application is enabling for the claimed invention." *M.P.E.P.* § 2164.01(c). In view of the above, Applicants respectfully request that the Examiner reconsider and withdraw the enablement rejection under 35 U.S.C. § 112, first paragraph.

CONCLUSION

In view of the above, Applicants respectfully maintain that claims are patentable and request that they be passed to issue. Applicants invite the Examiner to call the undersigned if any remaining issues may be resolved by telephone.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: April 26, 2006

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